

Immune Deposits in Human Glomerulopathy

By Svend Larsen



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IMMUNE DEPOSITS
IN HUMAN
GLOMERULOPATHY

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Immune Deposits in Human Glomerulopathy

(Immunofluorescent microscopy study)

By Svend Larsen

COPENHAGEN 1981



*To work something out,
you have to work your way in,
but when you finally worked your way in,
you can't work it out.*

Storm P.

This thesis is a review based on the following articles:

Larsen, S.: Immunofluorescent microscopy findings in minimal or no change-disease and slight generalised mesangioproliferative glomerulonephritis.

Acta path. microbiol. scand. Sect. A 86: 531–542, 1978

Larsen, S.: Immune deposits in generalised mesangioproliferative glomerulonephritis.

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Larsen, S.: Glomerular immune deposits in kidneys from patients with no clinical or light microscopic evidence of glomerulonephritis.

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Larsen, S. & Brun, C.: Immune deposits in human glomerulopathy.

Acta path. microbiol. scand. Sect. A 87: 321–333, 1979

ABBREVIATIONS

C	Complement
EM	<i>Electron microscopy (microscopic)</i>
Fab	<i>Antibody binding fragment of immunoglobulin</i>
Fc	<i>Crystallisable fragment of immunoglobulin</i>
GBM	<i>Glomerular Basement Membrane(s)</i>
GN	<i>Glomerulonephritis</i>
HRP	<i>Horseradish peroxidase</i>
HSP	<i>Henoch-Schönlein purpura</i>
IF	<i>Immunofluorescence (Immunofluorescent)</i>
IFM	<i>Immunofluorescence microscopy (microscopic)</i>
Ig	<i>Immunoglobulin</i>
LM	<i>Light microscopy (microscopic)</i>
MPGN	<i>Membranoproliferative glomerulonephritis</i>
PAP	<i>Peroxidase – antiperoxidase</i>
SD	<i>Systemic disease</i>
SED	<i>Subendothelial deposits</i>
SLE	<i>Systemic lupus erythematosus</i>

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I INTRODUCTION

In recent years development in the treatment of both acute and irreversible terminal renal failure has led to increasing interest in, and need for, more precise clinical diagnosis. This has resulted in a remarkable advance in the investigative methods used to study the morbid anatomy and immunopathology of renal diseases so that a more precise diagnosis is obtained. This applies of course to all large groups of kidney disease but particularly to glomerulonephritis (GN).

In GN, haematuria, proteinuria and nephrotic syndrome are the most common clinical presentations of either functional changes or damage in glomerular tissue. Uraemia is seen relatively rarely in the acute phase, but is characteristic of cases with a protracted course, where the condition can lead to irreversible terminal uraemia. Clinical observations do not permit any definitive classification of GN but much new knowledge has appeared in this field. This is based partly on the introduction of the percutaneous renal biopsy technique and the use of both light and electron microscopy and immunomicroscopy, and partly on immunological research including both human and experimental studies.

Using a refined immunofluorescence microscopic (IFM) technique it has been the aim of the studies referred to in this thesis to elucidate whether the occurrence of immune deposits in glomeruli always coexists with the clinical picture, GN as diagnosed on light microscopy (LM), and how far it is possible for IFM to be used as a basis for a definitive classification of GN.

II GLOMERULOPATHY OF IMMUNOLOGICAL ORIGIN

Experimental studies and clinical observations at the beginning of this century aroused suspicion that the glomerular lesions could be caused by two different immune mechanisms.

EXPERIMENTAL STUDIES

Antiglomerular basement membrane (GBM) nephritis

Lindeman (1900) demonstrated the first so-called nephrotoxic serum nephritis in rabbits by injecting them with serum from guinea-pigs immunised with rabbit kidney extract. Further studies by Masugi (1933–34) and by Smadel & Farr (1937) and Smadel (1937) showed glomerular lesions, as well as clinical and functional relationships, which were to a certain extent analogous with human GN.

Another model was introduced by Steblay (1962). Sheep were immunised with GBM from different species (including human) in Freund's adjuvant. This induced autologous antibody formation directed against the animals' own GBM. This phenomenon can be presumably accounted for because of some similarity between the antigenic determinants in the basement membranes of different animals (cross re-activity). Following nephrectomy it was possible to demonstrate circulating antibodies in the blood directed against GBM (anti-GBM antibodies) (Steblay 1965) and so the term anti-GBM nephritis was introduced.

Immune complex nephritis

In 1911 Pirquet advanced his hypothesis about immune complexes as a cause of nephritis. He drew attention to the similarity between the latent period for development of nephritis in scarlet fever (Schick 1907) and that in serum sickness in both rabbits and humans following injection of horse serum. This consistency, in addition to precipitation experiments on the serum of animals following injections of horse serum, led him to conclude: that serum sickness, and nephritis as part of this, was

a result of "toxic bodies" (now called immune complexes) formed by interaction between the administered antigen (horse serum) and the antibodies produced during the latent period.

Evidence that immune complexes are a potential factor in inducing experimental GN was reported in 1959–60 (McCluskey et al. 1959, McCluskey et al. 1960, Benacerraf et al. 1960). By giving intravenous injections of pre-formed immune complexes in mice and rats respectively, glomerular damage was induced in 50–90% of the animals, dependent of the antigen-antibody system used.

Both the type of lesions as well as their degree of severity varied both within the same experimental groups and between the groups.

Such complex formation between antigen of non-glomerular origin and specific antibodies can also take place in circulating blood and lead to the formation of circulating "nephritogenic" antigen-antibody complexes (immune complexes). These can then deposit in the glomerular tissue (Dixon 1968) and thus the term "immune complex nephritis" was introduced.

On the basis of numerous experimental studies an enormous amount of literature has appeared in the last 20 years on the details surrounding the sphere of activity of these two immune mechanisms and their significance in the development of GN (reviewed by Unanue & Dixon 1967, Cochrane & Koffler 1937, Wilson & Dixon 1974a). It is evident from this that in GN both types of immune mechanisms activate the same mediator systems, resulting in immunological damage to the glomerular tissue.

An experimental model of immunocomplex glomerulonephritis induced by "in situ" immunocomplex formation in GBM has recently been proposed (Batsford et al. 1980). It was demonstrated that a part of the injected (exogenous) antigen "planted" within the GBM can act as a target for specific antibody molecules inducing immune complex nephritis. Therefore it is possible that circulating soluble immune complex deposition is not the sole immune pathogenetic mechanism which can induce GN.

Mediator systems

When concentration of immune complexes occurs in glomeruli the classical pathway of the complement (C) system is activated and C_{1q} formation leads to C_3 activation. This results in the formation of chemotactic substances which cause neutrophil granulocytes to migrate to the

place where the immune complex is localised. The neutrophil granulocytes release lysosomal enzymes which damage the surrounding tissue. However, in spite of antigen-antibody deposits being present in the capillary loops it has not always been possible to demonstrate C_3 . It is therefore presumed that other pathogenetic mechanisms can also be the cause of the glomerular damage, where the deposited immune complexes are possibly secondary accumulations in the damaged tissue. However, this does not exclude the possibility that such deposits contribute to the continued maintenance of the lesions.

Autologous glomerulonephritis

A third immunopathogenetic mechanism causing glomerulopathy was reported by Heymann et al. (1959). With prolonged immunisation of rats with autologous kidney extract a selective thickening of the GBM was produced with accompanying proteinuria. Whether this membranous nephropathy is caused by the deposition of circulating immune complexes in glomeruli, or whether there is some question of an *in situ* formation of immune complexes in the basement membranes of the capillary loops, is controversial. The antigen in these immune complexes may be a component of the brush border of the tubular epithelium (Grupe & Kaplan 1969). Both the antigen and antibody directed against it have been demonstrated in the circulation (Grupe & Kaplan 1969, Sugisaki et al. 1973) as well as in the glomerular deposits (Edgington et al. 1968), which point towards an immune complex nephritis. However, more recent investigations suggest the antigen-antibody complexes are formed locally in the basement membranes (Couser et al. 1976, Salant et al. 1979). Whether this indicates that some independent, possibly autoimmune, mechanism is involved here, or whether the mechanisms already mentioned above are operative in this type of glomerulopathy, is not clear. The demonstration of mononuclear cells in the interstitial tissue in some cases of Heymann nephritis has further opened the possibility that cell-mediated mechanisms may also be involved (Sugisaki et al. 1973).

As the two main types of immune mechanisms involved with anti-GBM nephritis and immune complex nephritis, respectively, are capable of inducing the same type of glomerular lesions it is impossible on purely morphological grounds to determine which of the immune mechanisms is involved.

Glomerular lesions

Characteristic glomerular lesions induced by the two immune mechanisms have been reported by among others Mellors et al. (1955) and Dixon et al. (1961). The predominant types of lesion are glomerular hypercellularity of mesangial or endothelial origin. The damage can include all glomeruli (diffuse = generalised distribution) or only few glomeruli (local = focal distribution). The hypercellularity can include the whole glomerulus (global) or a part of the capillary tuft (segmental). Some lesions may be characterised by a more or less pronounced granulocyte infiltration where there may be foci of necrosis, fragmented nuclei, as well as hyalinised or structureless capillary walls. Damage can also occur in Bowman's capsule. This is characterised by proliferation of the epithelium of the capsule (crescents) whilst in other instances adhesions are seen between the parietal and visceral epithelium. In more chronic disease the lesions showed characteristic thickening of the basement membranes sometimes accompanied by lobulation of the capillary loops with obliterated glomeruli and scar formation.

Important factors involved in the induction and progress of glomerulonephritis

Experimental studies on GN have shown that both the potential ability to induce the lesions and the degree of their severity, as well as reversibility and chronicity, are conditional on a number of different immunological circumstances as well as physical and physiological factors. The following may be mentioned:

- 1) the quantitative amount of antigen and duration of effect of the antigen (Germuth et al. 1967a) as well as the induction properties of the antigen (Elling 1979).
- 2) the quantity and quality of antibody response (Masugi 1934, Dixon et al. 1961, Unanue & Dixon 1967).
- 3) valency of the antigen and the affinity of the antibody (Koyama et al. 1978).
- 4) size of the immune complexes (Germuth et al. 1972, Koyama et al. 1978, Germuth & Rodriguez 1973).
- 5) vasoactive amines and histamine (Cochrane & Hawkins 1968).
- 6) complement activation by immune complexes (Vogt & Kochem 1961, Unanue & Dixon 1964, Cochrane et al. 1968).

- 7) coagulation factors (Vassalli & McCluskey 1971).
- 8) haemodynamic conditions (Germuth et al. 1967b).
- 9) reduced phagocytic function of mesangium (Michael et al. 1967, Hoyer et al. 1976, Kijlstra et al. 1978).
- 10) "autoimmune disease" on a possible viral or genetic basis (Cochrane & Koffler 1973) or
- 11) spontaneous idiopathic development of GN (Lerner & Dixon 1968).

Demonstration of immune processes

Immunofluorescence microscopy (IFM)

Direct demonstration of immune processes involved in glomeruli in experimental GN was made possible when Coons and his colleagues introduced IFM for identifying antigens in tissue (Coons et al. 1941, Coons & Kaplan 1950). By means of this method it is possible from the appearance of the immune deposits and their localisation in glomeruli to differentiate between the various types of immune mechanisms which are involved in GN, provided that the glomerular structures are reasonably well preserved (Dixon 1968). Anti-GBM nephritis can therefore be identified by a bright continuous linear fluorescence in or along the glomerular basement membranes. This type of GN is therefore often termed "linear nephritis". Immune complex nephritis is characterised by a bright, mixed, fine and coarsely granular fluorescence localised both to the basement membrane of the capillary loops and mesangium. This is characteristic for GN seen in serum sickness (Germuth & Rodriguez 1973). In autologous GN = Heymann nephritis = membranous GN, a uniform finely granular fluorescence is seen solely along the basement membranes of the capillary loops (Salant et al. 1979).

Electron microscopy (EM)

EM studies of experimental GN have shown electron dense deposits along the endothelial side of the basement membranes of the capillary loops in the early phase of anti-GBM nephritis (Andres et al. 1963, Feldman et al. 1963). Later in the course of this type of GN these deposits are seen in a subepithelial position.

In immune complex GN electron dense deposits can be demonstrated in the subepithelial position (Andres et al. 1963, Feldman 1964) as so-called "humps" (Kimmelstiel et al. 1962). In the membranous type of GN (Heymann nephritis) somewhat smaller electron dense deposits are

seen regularly arranged along the epithelial side of the capillary loops (Feldman 1964, Salant et al. 1979).

Whether all these deposits which can be demonstrated by EM represent immune deposits or whether they largely consist of other material is not clarified with certainty at the present time (Batsford et al. 1980).

HUMAN GLOMERULONEPHRITIS

Knowledge of the morphological changes seen in kidney disease in human was formerly mainly based on post mortem investigations.

Needle biopsies

The introduction of the percutaneous needle biopsy technique by Perez Ara (1950) and Iversen & Brun (1951), for removing fresh samples of kidney tissue from patients with suspected or overt kidney disease opened new pathways for the study of glomerular diseases in humans. It therefore became possible to relate the morphological and immunopathological findings to the actual clinical picture. In addition, by means of re-biopsy, the morphological changes and immune deposits could be followed throughout the course of the disease.

The usefulness of the technique and the low risk of complications (Brun & Raaschou 1958, Kark et al. 1958) has led to its increasing use in nephrology and nephropathology (Wilson & Dixon 1974a).

Morphological and immunohistological evaluation

Morphological:

Criteria for a LM appraisal and classification of the morphological changes in the renal corpuscle in GN was published by Pirani & Salinas-Madrigal (1968) and Habib (1970). Generally speaking the different types of glomerular lesions do not represent different phases in the same disease. However, changes from one histological type to another have been reported during the course of the disease (Ginzler et al. 1974, Richet et al. 1974, Gill et al. 1977, Mahajan et al. 1978). The reproducibility of LM types of lesions which nowadays constitute the concept of GN is reasonably good, though there is some degree of unreliability in differentiating between, "normal glomeruli", "minimal change lesion" and mild mesangioproliferative GN (Black et al. 1970). This uncertainty in differentiating between these groups by ordinary LM can to some

extent be eliminated by LM quantitative counting of the nuclei in the different cellular parts of the glomeruli (Kawano et al. 1971, Bohle et al. 1974, Sørensen 1977). However, glomerular changes can be so slight that they cannot be recognised on conventional LM and so a classification of the glomerulopathy is only possible with supplementary IFM or EM.

On LM diseases or conditions with glomerular lesions which are of a focal nature can be mistakenly classified as either "normal" or "minimal change lesion" because damaged glomeruli are not represented in the biopsy. Such errors of diagnosis are possible if the biopsy contains only a few glomeruli and especially if the damaged glomeruli are localised to the corticomedullary area (Habib 1973).

In spite of dividing GN into different histological types there is no specific correlation between these types and the clinical picture (Tresler et al. 1969, Cameron 1975), and the glomerular lesion gives no direct information as to whether immunopathogenetic mechanisms have caused the lesion or which immune mechanism is involved.

Immunohistological

IFM has therefore been used increasingly in recent years, in parallel with morphological studies of kidney biopses, to identify the type of immune mechanisms involved which can be the pathogenetic cause of the glomerular lesions in GN. Using this method it has been shown that in many human kidney diseases it is possible to demonstrate glomerular deposits of immunoreactants as an expression of the involved immune mechanisms, which to a certain extent are analogous with those that can be demonstrated in experimental GN (Taft et al. 1958, McCluskey 1970, Dixon 1968).

It is estimated that immune mechanisms are responsible for about 80% of human GN (Wilson & Dixon 1974b). This can be primary (Michael et al. 1966, Burkholder et al. 1969) or secondary as part of a systemic disease such as systemic lupus erythematosus (SLE) (Dujovne et al. 1972, Agnello et al. 1973), Henoch-Schönlein purpura (HSP) (Urizar et al. 1968), and Goodpasture's syndrome (Duncan et al. 1965, Koffler et al. 1969).

The most commonly occurring type of GN is "immune complex nephritis".

"Linear nephritis" (anti-GBM nephritis), which is relatively rare, is reported with varying frequency in different studies: in France 0% (Berger et al. 1971), in Denmark 2% (Larsen & Brun 1979). A higher

frequency of linear nephritis (4–7%) is reported from USA (Wilson & Dixon 1974b). This rather high percentage of linear nephritis, however, is based on a selected material (Dixon, personal communication) and geographical differences are therefore hardly relevant.

Objections to drawing conclusions by analogy between human and experimental glomerulonephritis from glomerular immune deposits

The commonly accepted theory that the two main types of immune mechanisms are responsible for inducing most instances of human GN must be taken with some reservation, for:

- 1) the theory does not clarify how these two immune mechanisms can induce such widely different lesions in glomeruli when these are generally not different phases in the same glomerular disease.
- 2) the antigen in the presumed immune complex deposits in glomeruli is only rarely demonstrated, and then with most confidence in the case of DNA in SLE (Koffler et al. 1967).
- 3) a demonstration of C_3 does not necessarily indicate activation by immune complexes, for C_3 can be activated by an alternative pathway (the properdin system) (Ruddy et al. 1972) and the glomerular lesions cannot be differentiated from those induced by immune complexes.

In addition, in more recent years there have been reports of glomerular deposits of immunoglobulins (Ig) and/or C_3 in glomeruli in diseases which are reputedly of non-immunological origin, e.g. cystinosis and hereditary nephropathy (Berger et al. 1971), diabetic nephrosclerosis (Thomsen 1972, Hyman et al. 1973), pyelonephritis (Beregi et al. 1974) and bilateral cortical necrosis (Mahieu et al. 1972).

Furthermore, glomerular deposits of Ig or C_3 have been demonstrated in glomeruli in a number of conditions without either clinical or histological evidence of GN, e.g. in living kidney donors (Velosa et al. 1976), in extrarenal cancer (Constanza et al. 1973, Pascal et al. 1976, Sutherland et al. 1974a) as well as in kidneys examined at post mortem (Sutherland et al. 1974b). It is possible that these deposits represent a clearance of non-nephritogenic immune complexes or aggregated Ig, in a similar fashion to clearance mechanisms in the rest of the body's reticulo-endothelial system (Aschoff 1925).

Thus, at the present time it is clear that glomerular immune deposits in kidney biopsies taken from patients with GN are not necessarily con-

nected with "the disease GN". Greater caution must therefore be exercised in drawing conclusions by analogy about the importance of demonstrated immune mechanisms in the induction and progress of human GN which are based on experimental experience with animals.

III IMMUNOLOGICAL MECHANISMS (as related to *Human Glomerulonephritis*)

This section aims at giving a short account of the immunological phenomena which may be important in evaluating the IFM findings in human GN.

The immune system consists of immunocompetent cells which originate from stem cells in the bone marrow and are characterised by a fundamental division into two populations. One group consists of T lymphocytes which after maturation in the thymus are responsible for cell-mediated immune reactions (cell-mediated immune response). The other population consists of B lymphocytes and in humans, after their maturation in the organ analogous to the bursa, these develop into antibody-producing plasma cells. These are responsible for antibody-mediated immune reactions (humoral immune response).

CELL-MEDIATED IMMUNITY

Cellular immunity is presumed to contribute only to a slight extent to the production of human GN. This is in contrast with non-immunised allograft rejection where cellular immunity plays a dominant role (Hume 1971, Russell & Winn 1970). However, stimulation of the immune system by antigens containing more than two antigenic determinants can possibly initiate both a cellular and humoral response because of some degree of co-operation between T and B lymphocytes (Roitt 1973).

A hypothesis involving organ-specific cellular immunity in GN was put forward by Bendixen (1968). In patients with active (but not morphologically well-defined) GN, he demonstrated an inhibition of leucocyte migration in the peripheral leucocytes in a test system where the antigen was foetal kidney homogenate. Later studies have supported the hypothesis in that cellular immunity has been reported in progressive GN (Zabriskie 1971, Fillit et al. 1978) and shown it could accompany the humoral response in certain forms of proliferative GN (Mahieu et al. 1972) and be involved in minimal change nephrotic syndrome (Eyres

et al. 1976, Mallick 1977). Cell-mediated immune reactions may therefore be considered responsible for some of the approximately 25% of GN where no deposits of Ig/C₃ are demonstrated in glomeruli on IFM (Larsen 1978a, Larsen & Brun 1979). Similarly it is not possible to exclude that immunosuppressive therapy is directed against this arm of the immunological response in GN (Larsen 1978b).

A defect in the cell-mediated immune response has been demonstrated in patients with SLE in the active phase (Lockshin et al. 1975, Horowitz et al. 1977, Rosenthal & Franklin 1975). And indeed, it is in this very group of patients that the clearest evidence exists for GN of immunological origin (Koffler et al. 1967). It is therefore not possible to exclude that a defect in the cell-mediated immune response is a contributing cause for an altered humoral response, with a greater production of immune complexes resulting in GN of immune complex origin.

However, the significance of cell-mediated immune mechanisms in the production of human GN, whether this is primary or secondary to systemic disease, is not clarified.

HUMORAL IMMUNITY

The deposition of circulating antigen-antibody complexes in glomeruli with resulting complement activation is nowadays presumed to be the most important cause of human GN. In 75–85% of cases glomerular deposits of Ig/C₃ are demonstrated (Larsen & Brun 1979, Wilson & Dixon 1974b, Hyman et al. 1973).

The antigen

The antigen in involved antigen-antibody systems (immune complexes) in human GN is unknown with few exceptions, and only demonstrated with certainty in the case of DNA in immune complexes eluted from kidney tissue in SLE (Koffler et al. 1967). However, antigens along with Ig and C₃ have been demonstrated in glomeruli in a number of bacterial, viral and plasmodial infections, accompanied by either GN or nephrotic syndrome (McCluskey 1974, Wilson & Dixon 1974a, Combes et al. 1971, Brzosko et al. 1974, Tourville et al. 1976, Friedberg et al. 1978, Sato et al. 1979). Similarly, glomerular deposits of tumour antigen, along with Ig and C₃, have been demonstrated in extrarenal cancer (Constanza et al. 1973) and tubular antigen with Ig/C₃ has been reported in idiopathic nephropathy (Miyakawa et al. 1976).

Demonstration of antigen along with Ig and C₃ gives strong support to the view that this glomerulopathy is of immune complex origin. However, studies (especially of GN related to streptococcal infection) have shown that antigen and Ig or C₃ are not always localised to the same place in the glomeruli (reviewed by Zabriskie et al. 1973). Only elution of the deposits in the kidney tissue can therefore be proof that the deposits represent immune complexes. Therefore, no definite conclusion can be reached about whether some types of antigens with a direct toxic effect are a cause of glomerular lesions, where the damaged tissue then acts as an antigen and thus leads to production of autoantibodies directed against the glomerular tissue components, or, whether perhaps there is a combination of both. Details in the make-up of the antigen are still not clarified and this must be presumed to be of decisive significance in both the induction, as well as in determining the acute or chronic nature of the course of the disease in GN. No specific correlation between the antigens known at the present time and the morphological types of lesions or deposited Ig classes has been demonstrated.

Immunoglobulins

Circulating antibodies belong to the special group of plasma proteins called Ig. From the structure of their crystallisable fragment (Fc) they can be subdivided into five classes termed Ig (G, M, A, D, E). Their specificity for antigens is attached to the antigen binding fragment (Fab).

For details about the composition, structure and specific data and characteristics of the immunoglobulins the reader is referred to Roitt (1973) and Sternberger (1979).

The half-life for the turn over of the five classes of Ig in the circulation (Zollinger 1978) is: IgG = 25 days, IgM = 5 days, IgA = 7 days, IgD & IgE less than 3 days. The turn over for deposited Ig in humans is not known.

A short mention is made below of the individual classes of Ig and their occurrence in human glomeruli.

Immunoglobulin G (IgG)

IgG is a monomer, has a molecular weight of 150,000 (7s) and makes up 80% of the total Ig concentration. IgG can be subdivided into four subclasses of which G1, G2 and G3 can activate (bind) C in contrast with G4 which does not activate C. Studies of deposits of these four subclasses in glomeruli in GN (Lewis et al. 1970) have shown that G3

predominates when GN is secondary to SLE, whilst G4 predominates in linear nephritis. Deposits of G4 can thus be one explanation of why C3 cannot be demonstrated along with IgG in approximately 25% of cases of linear nephritis (Wilson & Dixon 1974a). It must therefore be presumed that the glomerular lesions could be induced by other mechanisms than that mediated by C.

IgG can occur in all LM types of GN, though with varying frequency (Larsen & Brun 1979, tab. 2, Hyman et al. 1973).

In GN, including minimal change lesion, glomerular deposits of IgG occur in about 50% of cases, according to Zollinger (1978), whilst the author's own studies have shown a little higher incidence with 62% in primary GN and 74% in GN secondary to systemic disease (Larsen & Brun 1979).

The occurrence of deposited IgG in glomeruli in non-glomerulonephritic nephropathy is reported as 15% (Zollinger 1978) and 29% (Larsen & Brun 1979, tab. 2). Furthermore, it can be demonstrated in 16% of persons without either clinical or LM evidence of glomerulopathy (Larsen 1979), and demonstrated as non-specific IgG in eluates from kidney tissue in older people with no kidney disease (Wesenberg & Tönder 1978). One must therefore conclude that glomerular deposits of IgG which are demonstrated in GN are not always related to »the disease GN«.

Immunoglobulin M (IgM)

IgM has a molecular weight of 900,000 (19s) and it is found predominantly as a pentamer with a strong binding capacity for C. Antibodies produced in the primary response to antigen stimulation are mainly IgM, though this switches to IgG in prolonged or recurrent stimulation.

IgM reacts with antigenic determinants on blood cells, bacteria and viruses and in addition can occur as heterophile antibodies when they are directed against the Fc portion of IgG. The pentameric construction, with many binding sites for antigenic determinants, can be a contributory cause for IgM reacting non-specifically, and to a greater extent than the monomeric classes of Ig, with antigen determinants which differ from those stimulating the IgM production. Deposits of IgM can occur in all types of GN (Larsen & Brun, 1979, tab. 2). As IgM is the first Ig response to primary antigenic stimulation, it might be expected that IgM would be the most common finding in glomeruli in acute GN. This is not the case, for IgG is demonstrated most frequently in renal biopsies

from patients with acute GN (Verroust et al. 1974b). Furthermore, in biopsies from patients with GN of shorter duration than three months, there is a significantly reduced incidence of demonstrated IgM in comparison with that which can be seen in biopsies taken later in the course of the disease (Larsen & Brun 1979, tab. 4). IgM/C₃ occur with almost the same frequency in primary GN (44%) and GN secondary to systemic disease (47%) (Larsen & Brun 1979) and Zollinger (1978) reports a frequency of 55% in GN as a whole. The highest incidence reported is 80% in idiopathic nephrotic syndrome with minimal change lesions or mesangioproliferative GN and focal sclerosis (Michael et al. 1973). Aggregated IgM can bind C₃ (Christian 1970) and, depending on the size of the complexes (Cochrane & Hawkins 1968), and the serum level (Kijlstra et al. 1978), this can precipitate from the circulation either solely in the mesangium, or in both the mesangium and capillary loops. Furthermore, in recent years there have been reports of IgM being deposited in glomeruli, with or without C₃, in non-glomerulonephritic nephropathy (Berger et al. 1971, Hyman et al. 1973, Tribe et al. 1979). The frequency of such deposits has been reported as 40% (Zollinger 1978) and 22% (Larsen & Brun 1979, tab. 2). Similarly, IgM has been demonstrated in 14% of people without clinical or LM evidence of GN (Larsen 1979) and in 33% (2 out of 6) in kidney donors (Michael et al. 1973). The relatively similar incidence of glomerular deposits of IgM in GN (whether this is primary in origin or secondary to systemic disease) and non-glomerulonephritic nephropathy, when considered together with the relatively rare occurrence of IgM in acute GN, strongly suggest that these deposits are a secondary phenomenon. In this situation IgM is secondarily deposited in damaged (Tribe et al. 1979) or functionally altered glomeruli, and thus has no essential importance in *the induction* of human GN. The possibility that the glomerular deposits of IgM may be responsible for maintaining the pathological processes in the glomeruli cannot be excluded. Thus, IgM deposits may well be of importance to both the nature of the course of the disease and the prognosis. It therefore seems likely that the demonstration of glomerular deposits of IgM (Rumpelt & Thoenes 1973) can be used as a prognostic indicator to separate different categories of patients with nephrotic syndrome and the same type of glomerular lesion, as reported by Cohen et al. (1978).

Therefore, the demonstration of IgM deposited in glomeruli, with or without C₃, has a limited value in separating GN from non-glomerulonephritic nephropathy but can be an indicator of more "chronic" lesions in glomeruli.

Immunoglobulin A (IgA)

IgA is a monomer with molecular weight 150,000 (7s) making up 85–90% of serum IgA. The remaining 10–15% is composed of dimeric, trimeric and tetrameric molecules (in order of decreasing concentration). In secreters dimeric IgA is coupled to a secretory piece (molecular weight 390,000 (11s)). It is known that secretory IgA has antibody specificity for bacterial antigens, viruses and toxins where it functions as a substrate for extracellular enzymes (IgA protease) reviewed by Plaut (1978). By agglutinating these it prevents them binding to the surfaces of cells. Similarly, it has been shown that IgA binds to neutrophil granulocytes and prevents neutrophil chemotaxis. More precise details of the biological function of secretory IgA *in vivo* are not clarified.

Secretory IgA cannot be demonstrated (Lowance et al. 1973) or is only rarely demonstrated in GN (McCoy et al. 1974) and is presumed to play no pathogenetic role in producing GN.

IgA can occur in all types of GN though with different frequency (Hyman et al. 1973).

Whether the glomerular deposits of Serum IgA in GN represent the antigenic or the antibody portion in deposited “immune complexes”, or whether it is aggregated IgA, is not clarified. The reported incidence of IgA in GN as a whole (including minimal change lesion) ranges from 35% (Zollinger 1978, Lowance et al. 1973) to 43% (Larsen & Brun 1979). However, it occurs most frequently in GN secondary to SLE (55–60%) (Larsen & Brun 1979, Hyman et al. 1973) and HSP (83–88%) (Hyman et al. 1973, Larsen & Brun 1979).

In GN, IgA and IgA – IgG deposits are significantly different from other deposits. They are more commonly localised to the mesangium (Larsen & Brun 1979, tab. 4), and eluate from kidney tissue containing IgA (nephrectomy specimens) has shown a slight but specific reaction with intraglomerular components in normal renal tissue (Lowance et al. 1973). Therefore, the possibility that IgA can be an antibody where the antigen is either the mesangium itself, or antigenic components in the mesangium, cannot be excluded. On the other hand IgA is commonly deposited together with IgG (Berger 1969, Berger et al. 1971, Druet et al. 1970, Lowance et al. 1973, Hyman et al. 1973, McCoy 1974, Larsen & Brun 1979, tab. 4) so that IgA – IgG deposits in glomeruli possibly represent antigen-antibody complexes formed *in situ*, with IgA acting as antigen and IgG as the antibody component.

It is well known that aggregated IgA can activate C₃ and later C fractions (C₅–C₉) via the alternative pathway (Götze & Müller-Eberhard

1971), but in contrast it has only been recently demonstrated that monomeric IgA (Fc portion) can activate C_3 via the classical pathway (reviewed by Gärtner et al. 1979). Therefore, there is now an explanation available of why IgA/ C_3 can be deposited with or without properdin and with or without C_{1q} and C_4 . On the other hand there is no explanation of what IgA/ C_3 represents in those cases where neither properdin, C_{1q} , nor C_4 are seen (Larsen 1978a, Larsen & Brun 1979).

Mesangial IgA – IgG nephropathy was originally defined by Berger (1969) as an independent, well-defined group where the aetiology was unknown. It was characterised by intercapillary deposits of IgA, IgG, and C_3 , with focal glomerular changes on LM (which were, however, not uniform) and a relatively uniform clinical picture with persistent haematuria (slight proteinuria) and a protracted course (3–17 years). One of the patients observed, ended up with irreversible renal failure, nephrectomy and transplantation, with residual haematuria. In the graft biopsy taken one year after transplantation mesangial deposits of IgA, IgG and C_3 were demonstrated.

In the years following this, numerous studies have focused on IgA deposits in GN (reviewed by Zollinger 1978, Gärtner et al. 1979). Mesangial deposits of IgA or IgA – IgG were demonstrated in both “normal glomeruli”, “minimal change lesion”, as well as in focal and generalised proliferative GN and unclassifiable GN. These findings are in agreement with the author's own studies (Larsen 1978a, Larsen 1978b, Larsen & Brun 1979). The prognosis seems to some extent to be related to the morphological type of lesion (Levy et al. 1973).

However, mesangial deposits of IgA – IgG/ C_3 can be accompanied by severe proteinuria (Lowance et al. 1973, Larsen 1978b) and nephrotic syndrome (McCoy 1974) and is seen commonly in HSP and more rarely in SLE (Larsen 1978a). This is comparable to the many different reports on the incidence of IgA – IgG nephropathy which ranges from 2.1% (Hyman et al. 1973) to 22% (Druet et al. 1970). This suggests that patients with mesangial deposits of IgA, IgG and C_3 form a much more heterogenous group than Berger (1969) first supposed. This great variation in the incidence of IgA–IgG nephropathy in the different studies of GN may have resulted because the various studies included more types of histological lesions than earlier defined by Berger.

If IgA–IgG nephropathy can be regarded as an independent disease entity it is nevertheless only possible to separate it from other types of GN by using IFM. However, a precise diagnosis can only be made from

amassed information about morphology, immunopathological findings and clinical data.

Even though the role of IgA in human GN is not clarified, it seems that IgA is the very class of Ig demonstrated in glomerular deposits which correlates best with the LM diagnosis of GN. For IgA only appears in 10% (Larsen & Brun 1979) or less (Zollinger 1978) of patients with non-glomerulonephritic glomerulopathy and has not been demonstrable in patients without clinical or LM evidence of GN (Larsen 1979).

Immunoglobulin E (IgE)

IgE has a molecular weight of 200,000 (8s) and belongs to the group of Ig known as "topic" antibodies, or reagins, which are normally found in low concentration in serum. Included in the sphere of action of IgE are atopic and allergic reactions in which the Fc portion of IgE binds to mast cells and basophil granulocytes leading to release of histamine and vasoactive substances.

What immunopathogenetic role IgE antibodies play in human GN is not clear, and the demonstration of IgE in different studies of GN is controversial. Gerber and Paronetto (1971) demonstrated slight glomerular deposits of IgE in idiopathic nephrotic syndrome with morphological changes corresponding to "minimal change lesion". In consecutive kidney biopsies from 146 patients McPhaul et al. (1974) demonstrated glomerular deposits of IgE in 22 patients (15%) where the morphological changes represented several different types of glomerular lesions and where only occasional patients had nephrotic syndrome and none showed any evidence of atopy or allergic symptoms.

Similar results were noted by Zollinger (1978). In a study of 146 renal biopsies he demonstrated IgE in 20% of them including both patients with GN secondary to SLE as well as mesangial IgA-IgG nephropathy, but IgE did not appear in "minimal change lesion". In other studies including patients with specific pollen sensitivity (Cameron 1972), nephrotic syndrome (Michael et al. 1973) and the author's own investigations, IgE could not be demonstrated apart from in one single instance.

This discrepancy in the demonstration of IgE in different studies cannot be explained with any certainty and furthermore no specific relation to GN and/or clinical symptoms has been demonstrated. Similarly the prognostic importance of the presence of IgE is not clarified. One reason for IgE being demonstrated with such a low frequency may be because

of the widespread use of IFM using the direct technique, for it is known that IgE can be demonstrated almost twice as frequently using the indirect technique (Tarantino et al. 1974).

Immunoglobulin D (IgD)

IgD is a monomer with a molecular weight of 180,000 (7s) and a low serum concentration. The physiological role of IgD and the immunopathogenetic context in which IgD is involved *in vivo*, is unclarified. IgD (reviewed by Tarantino et al. 1974) does not bind C, but does present some antibody activity and has been shown to be involved in different situations such as: antinuclear antibodies, antithyroid antibodies, antibodies against diphtheria, toxin, penicillin and insulin. In all of these cases the serum also contained other classes of Ig which were specifically directed against the antigen concerned. It may therefore be presumed that the Fab portion of IgD is identical, or almost identical, with the Fab fragment of other classes of Ig. Studies of IgD in GN have only been reported in a few instances and among these is the study by Tarantino et al. (1974). Granular deposits of IgD were demonstrated in glomeruli in a number of different morphological types of glomerular lesions, mainly in chronic nephropathy in patients with GN secondary to SLE, membranous nephropathy and membranoproliferative GN (MPGN). IgD was always found together with other classes of Ig and did not differ in IFM appearance from these. The fact that there is an association of antigens between IgD and the other classes of Ig, and that IgD is always deposited in glomeruli along with other classes of Ig suggests that IgD and other classes of Ig compete for antigenic determinants. Thus it is possible that fewer molecules of the classes of Ig, IgG, IgM and IgA, are able to bind to the antigen, resulting in a low fluorescent intensity for the class of Ig concerned. Linear deposits of IgD, together with IgG have been demonstrated in linear nephritis where only the IgG fraction in serum was reactive against GBM (Katz & Pruzanski 1975).

In the author's own study of 110 renal biopsies from patients with GN (Larsen 1978a, Larsen 1978b) glomerular deposits of IgD were demonstrated in two cases, one MPGN (without systemic disease) and one "normal glomeruli" (in myelomatosis classified as a myeloma of IgM type). In both instances IgD was deposited together with IgA.

Comparable investigations of the direct and indirect technique for demonstrating IgD have shown that on the whole it was only possible to demonstrate IgD by the indirect technique (Tarantino et al. 1974).

As the direct technique is the one mainly employed in nephropathology this may be the reason for IgD being demonstrated so rarely in GN. On the other hand there is practically no difference in the sensitivities of the two methods in demonstrating IgG, IgM and IgA. At the present time the significance of IgD in GN is not clarified and further research is needed.

Mediator Systems

Complement/properdin

The complement/properdin system (Ruddy et al. 1972, Müller-Eberhard 1974) consists of a number of serum proteins of enzymatic nature, which together with various C fractions and inhibitors forms a functional unit. The system is an integral part of the body's defence mechanisms and functions as an effector pathway for both the coagulation system, and as a biocidal mechanism for eliminating bacteria, viruses and other foreign elements in tissue. The system consists of three functional components; *two pathways for activating C3*, the classical and alternative pathways, and a *common effector pathway* through C_5-C_9 which is responsible for the pathobiological activity of the system.

Classical pathway

The classical pathway consists of C_{1q} , which following splitting of C_4 and C_2 forms C_3 convertase ($C_{4,2}$) which activates C_3 . Activation of this classical pathway can be initiated by antigen-antibody complexes, where the antibody part can be the subclasses IgG₁, IgG₂ and IgG₃, or IgM and IgA (IgG₄ does not activate C).

Alternative pathway

The alternative pathway was first described by Pillemer et al. (1954). This by-passes C_1 , C_2 and C_4 and activates $C_{4,2}$ directly via the "properdin system". This pathway can be activated *in vitro* in fresh human serum by adding complex polysaccharides such as zymosan and inulin (Verroust et al. 1974a), endotoxin and aggregated IgA and IgG.

Common effector pathway

Activation of both the classical and alternative pathways can initiate the common effector pathway which via the complement cascade C_5-C_9 is responsible for such pathobiological effects as: increased vascular permeability, chemotactic migration of "neutrophils", adhesion between "com-

plexes" and formed elements in the circulation, increased phagocytosis and lysosomal release (Koch 1978) from membrane-related neutrophils which can result in osmotic lysis and necrosis.

Complement/properdin profiles in human glomerulonephritis

IFM studies of kidney tissue, based on the identification of Ig and C₃, present a possible way of differentiating the two immunopathogenetic mechanisms which are usually accepted as a cause of glomerular lesions in GN (Dixon 1968). In addition, in recent years there have been reports of different C/properdin profiles in glomeruli (Verroust et al. 1974b, Westberg et al. 1971), of which there are several variants (Müller-Eberhard 1974) such as:

- 1) early (C_{1q}, C₄) and late C (C₃, C₅, C₆, C₇, C₈) accompanied by Ig.
- 2) granular deposits of C₃ and late C (C₅–C₈) together with properdin, without Ig, C_{1q} or C₄.
- 3) granular deposits of C₃ and late C in some glomeruli, whilst in other glomeruli there are deposits of Ig, C_{1q} and C₄.
- 4) linear deposits of Ig, but without C.
- 5) deposits of early and late C (C₁–C₈), along with properdin.

Regarding 1) This is characteristic of immune complex nephritis and linear nephritis (Dixon 1968); both are activated via the classical pathway.

Regarding 2) Presumed to be a non-immunopathogenetic mechanism with activation via the alternative pathway. This way of activating C₃ has been recently demonstrated in serum (kidney tissue was not studied) in Gram negative bacteraemia leading to shock (Fearon et al. 1975).

Regarding 3) Possibly simultaneous or consecutive activation of the alternative pathway induced by immune complexes or perhaps aggregated Ig. This can also be seen in MPGN with subendothelial deposits (SED) (Habib et al. 1975).

Regarding 4) Rare: possibly represents an immunological C-independent type of GN, but can also be secondary deposition (insudate) in damaged basement membranes in the capillary loops.

Regarding 5) Activation via both the alternative and classical pathway. Characteristic of GN secondary to SLE (Ruthfield et al. 1972, Hunsicker et al. 1972), and most pronounced with activity of the kidney condition (Schur & Sandson 1968, Koyama et al. 1977).

Hypocomplementaemic MPGN with glomerular deposits of properdin/ C_3 (Westberg et al. 1971) and reduced serum levels of C_3 /properdin and normal C_4 levels (Hunsicker et al. 1972) has suggested there is a special type of GN activated via the alternative pathway. However, glomerular deposits of IgG and C_{1q} appear in only 50% of the cases (Westberg et al. 1971) so that this group is more heterogeneous than the properdin deposits in glomeruli and serum analyses suggest. It was later shown (Habib et al. 1975) that MPGN can be subdivided into two types based on morphological differences seen at EM: one with SED and one with intramembranous deposits. Using IFM in MPGN with intra membranous deposits, glomerular deposits of C_3 on its own are demonstrable and there are low serum levels of C_{1q} , C_4 and C_3 . This lack of demonstration of properdin in glomeruli does not seem indicative of alternative activation. MPGN with SED must still be presumed to represent a heterogeneous group. In spite of differences in morphology and C/properdin profiles in glomeruli these two groups cannot be separated clinically and the prognosis is the same for the two types (Cameron et al. 1973, Davis et al. 1978). Whether hypocomplementaemia in MPGN can be caused by a non-Ig gammaglobulin termed C_3 nephritis factor (C_3 NeF) (Faeron 1976), which expresses its function via the alternative pathway, is under debate.

Demonstration of C_3 without C_{1q} and/or C_4 in glomeruli is often used as an indicator of C_3 activation via the alternative pathway (Verroust et al. 1974b).

However, there is no corresponding parallel between deposits of C_{1q} and C_4 . It is therefore necessary to test for each component. In GN a significant difference is seen in the percentage ratio of demonstrated C_{1q} and/or C_4 in kidney tissue in comparison with the number of kidneys tested. In generalised proliferative GN this ratio varies from 62% (Verroust et al. 1974b) to about 26% (Zollinger 1978) and 23% in the author's own investigations (Larsen 1978b). As the lack of demonstration of C_{1q} and C_4 is supposed to be an indication that C_3 is activated via the alternative pathway then this mechanism should be operative in 38–77% in GN. This cannot be true as in many of these instances properdin is also not demonstrated, in spite of the lack of C_{1q} and C_4 . The absence of C_{1q} and C_4 is therefore not an unequivocal indicator of activation of C_3 via the alternative pathway. Therefore, the demonstration of properdin factors in glomeruli is a more certain indication of C_3 activation via the alternative pathway. A contributory cause for the lack of demonstration of C_4 may be the appearance of a " C_4 -binding pro-

tein" which, with its specific binding affinity to C_4 , is a block to anti- C_4 conjugates binding to C_4 in glomeruli (Scharfstein et al. 1978). Similarly, it is not possible to exclude that some solitary deposits of C in glomeruli are caused by non-nephritogenic complexes coated with C_3 depositing from the circulation. This may be the case because in human glomeruli, both C_3 receptors (Pettersson et al. 1978) and Fc receptors (Mizoguchi et al. 1978) have been demonstrated, though the precise site of these receptors is not known.

A direct toxic effect of C_3 has not been demonstrated as cause of the glomerular lesions in humans.

Experimental studies have shown that systematic activation of C_3 via the alternative pathway does not directly cause glomerular damage (Verroust et al. 1974a, Simpson et al. 1978). The importance of C to glomerular damage is therefore still not clear. The possibility of demonstrating C /properdin profiles in kidney tissue, seen in relation to the respective components in the circulation, may be expected to give further insight into the understanding of the probably many different pathways which underlie activation, and thus produce a better understanding of the course of the disease and prognosis of GN.

Fibrinogen/fibrin

Fibrinogen has a molecular weight of 340,000, is a dimer and makes up that part of the plasma which forms a basis for the body's coagulation processes. Intravascular coagulation is mediated by many serum factors (Montigel 1962) which following proteolytic splitting lead to fibrin formation. With both the breakdown of fibrinogen (primary fibrinolysis) and fibrin (secondary fibrinolysis) fibrin degradation products are formed which consist of antigenic fragments (D and E fragments). Qualitative and quantitative analyses of these can give a general outline of in vivo and in vitro fibrinogeno-fibrinolysis (Gormsen & Feddersen 1973). Both experimental studies and clinical observations (reviewed by Vassalli & McCluskey 1971, Kincaid-Smith 1975, Wardle 1974) have shown that intravascular coagulation with fibrin deposition in glomeruli can be a factor in causing glomerular damage. These can be correlated to an elevated serum level (Clarkson et al. 1971) and increased urinary excretion of fibrin derivatives (Clarkson et al. 1971, Dotremont et al. 1973).

Irrespective of the cause of systemic intravascular coagulation the kidneys can be a target organ: thrombi in arterioles and capillary loops

can in themselves lead to glomerular damage. The deposits of fibrin in these conditions are often of irregular shape (granular to linear), localised in the lumen of the capillary loops (segmental), and in the intima of arterioles (Gervais et al. 1971, Heptinstall 1974b), or in the mesangium and walls of the capillary loops (segmentally) and intramurally in the vessels (Feldman et al. 1966).

Fibrin deposits in glomeruli can appear in all types of GN (including "minimal change lesion") (Larsen 1978a, Larsen & Brun 1979) and the concentration of antigen-antibody complexes in glomeruli (Vassalli & McCluskey 1971, Borrero et al. 1973) with activation of C can be a cause for deposition of fibrin in glomeruli.

The author's own studies indirectly support this supposition as in patients with GN there is a significant reduction in the incidence of fibrin in glomeruli in patients without deposits of Ig and/or C₃ (Larsen & Brun 1979, tab. 4).

However, fibrin is demonstrated in glomeruli where non-immunological mechanisms are operative with activation of C₃ via the alternative pathway (Müller-Eberhard 1974), in acute and chronic GN with hypocomplementaemia (Westberg et al. 1971). Fibrin deposition without Ig or C₃ being present is, however, a characteristic finding in extracapillary crescents in extracapillary GN (Lewis et al. 1971, Olsen et al. 1974, Lawrence 1973, Stilmant et al. 1979), and thus inflammation or damaged tissue in itself can initiate fibrin deposition.

The fibrin deposits in glomeruli in GN were seen most commonly to be finely or coarsely granular or discontinuous and linear. The deposits are localised in capillary loops (most often subendothelially) and the mesangium, but can also be seen solely in the mesangium or solely along the capillary loops (Larsen 1978a, Larsen 1978b).

Glomerular deposits of fibrin also occur in a number of non-glomerulonephritic conditions such as myelomatosis (Ward & Preston 1974, Thomsen 1972), diabetes mellitus (Farquhar et al. 1972, Thomsen 1972), scleroderma and malignant hypertension (Kincaid-Smith 1975), cortical necrosis (Heptinstall 1974a), pyelonephritis (Beregi et al. 1974) and extrarenal cancer (Pascal et al. 1976).

It is therefore possible to be inclined to the view that glomerular deposition of fibrin can be of pathogenetic significance in human GN, but at the present time there is no evidence that this is the case, and the deposits can be a secondary event following damage or functional changes in glomeruli. Therefore, at the moment, it is impossible to draw any decisive conclusions about the pathogenesis of GN solely from the

fibrin deposits in glomeruli. Both the cause and the duration of the stimulus which has resulted in coagulation, and the effect of local and generalised fibrinolytic activity may have some influence on the likelihood of reversibility of the glomerular lesions. Fibrin deposits, in contrast to Ig and C deposits, can be identified both with IFM, EM and LM. Discrepancy in the incidence of demonstrated fibrin in different studies can be due to the method used. For example with EM fibrin can only be demonstrated from its periodicity (Feldman et al. 1966, Farquhar et al. 1972) and with histochemical staining methods other material besides fibrin may be stained (MacIver 1972). On the other hand immunomicroscopy (both IFM and immuno-EM) will bring both fibrinogen/fibrin and the antigenic intermediary fragments to light. Immunomicroscopy is therefore the method of choice for demonstrating "fibrin deposits".

IV IMMUNOHISTOCHEMICAL INVESTIGATION OF KIDNEY TISSUE

Immunohistochemical methods for demonstrating deposits of immunoreactants in kidney tissue have been used routinely for many years in many nephropathological centres in parallel with morphological investigations. The most used methods are generally based on the same fundamental immunological principle, namely: utilisation of "marked" (Fluorochrome or enzyme conjugated) immune sera with which the corresponding antigens in the tissue can be visualised, identified and localised. Immunopathological diagnosis on kidney tissue is based on an assessment of the appearance and localisation of deposits as well as identification of the deposited classes of Ig, complement fractions, fibrin/fibrinogen and other serum proteins. These are assumed to be an indication that immunoreactants are present in deposited antigen-antibody complexes.

Granular deposits of Ig and C along the basement membranes of the capillary loops and/or in the mesangium can be present in immune complex disease, whilst continuous linear deposits of Ig and C can be an expression for disease where circulating antibodies in the blood are directed against GBM (Dixon 1968). The composition of the deposited immunoreactants, their appearance and localisation can be a guide to diagnosis and classification in GN (McCluskey 1970, Larsen & Brun 1979). The following section takes a brief look at the use of immunohistochemical methods in nephropathology. For details about conjugating, purification of conjugates, and specificity, along with general methods for use of immunofluorescence (IF), the reader is referred to the review by Sternberger (1979) and Wick et al. (1978). Methods for using enzyme-conjugated antibodies have been recently reviewed by Sternberger (1979).

IMMUNOFLUORESCENCE

In 1941 Coons and his colleagues introduced the possibility of using IF. Since then, in the last 15 years, there has been refinement and develop-

ment of conjugates and optical systems which has resulted in the technique being widely used in biology, medicine and pathology. This wide usage has led to many different modifications of the procedure, and variation in the basic reagents. This, and the absence of any details about the strength of fluorescent conjugates and their specificity, has contributed to there still being no standard norms available for the method (Johnson et al. 1967, Wick et al. 1978). Confirmation of this state of affairs is clearly evident from the many reports on IF studies on kidney tissue where different laboratories clearly use their own modifications of the method. Other things being equal, this can be a contributory cause for the lack of agreement between results of studies carried out on comparable groups of patients.

Preparation of kidney tissue

The method most in use for preparing kidney tissue is rapid freezing, for which pieces of tissue about $2 \times 2 \times 6-8$ mm are very suitable. To freeze the tissue down, isopentane cooled by liquid nitrogen (-196°C) or dry ice (-79°C) can be used (Nairn 1969). Freezing with dry ice is easier to manage and most suitable for transporting. For transport long distances the use of a semipermanent mounting medium is recommended (Couser & Lewis 1974), for although the tissue is exposed to periodic warming-up to room temperature, both the morphology and antigenicity are preserved unaltered.

In the author's own investigation of kidney tissue frozen down by these two methods no difference was demonstrated in the morphological quality seen on conventional staining and LM (unpublished results). This is not the case for all types of tissue (Nairn 1969) or antigens (Arnold et al. 1975).

Autopsy material

Autopsy specimens of human kidney tissue can be treated in the same way as biopsies. If the autopsy material has been stored at a temperature below 10°C then Ig (G, M, A) can be identified with certainty up to 72 hours post mortem (Larsen 1979). This agrees with results on kidney tissue from rats (Seelig et al. 1975). C_{1q} and C_3 are less stable and can only be demonstrated with certainty up to 24 hours post mortem (Larsen 1979). C_4 studies can result in "false positive" findings, presumably

due to non-specific binding to autolysed or devitalised tissue (Larsen 1979).

Cryostat sections

Sections of the frozen tissue are cut on a cryostat which is a modified deepfreeze with a tightly fitting hinged window. A microtome, which can be operated from outside, is mounted in the freezer. Regarding technical details the reader is referred to Nairn (1969). The temperature in the refrigerated cabinet can be varied in most cryostats from -15°C to -25°C . The best cutting temperature for kidney tissue with a section thickness of $3\text{--}6\text{ }\mu\text{m}$ is -22°C to -24°C . Too high a temperature in the vicinity of the cryostat (summer, sunshine, warmth given off by the compressor of the cryostat) can lead to a rise in the freezer cabinet temperature to more than -20°C because of the compressor capacity being too low. The result of this is variation in section thickness and a deterioration in the morphology of the tissue. This makes it impossible, or at best difficult, to evaluate the localisation of the antigens in the tissue with precision. New types of cryostats (developed in recent years) differ from the conventional type by being equipped with a more powerful compressor. In these the freezer cabinet can be cooled down to -30°C . Others have further selective cooling of the object to be sectioned and cryotomes for cutting thin sections about $2\text{--}1\text{ }\mu\text{m}$.

The studies carried out by the author (Larsen 1978a, Larsen 1978b, Larsen 1979, Larsen & Brun 1979) were performed on this latter type of cryostat, the Leitz histocryotome[®]. The separate cooling of the cabinet and object make it possible to have an optimal cutting temperature so that consecutive sections $1\text{ }\mu\text{m}$ in thickness can be produced. However, when cutting such thin sections it is not possible to use the automatic equipment for slide and section transport which is housed in this histocryotome.

The thickness of the sections (with the cryotome set at $1\text{ }\mu\text{m}$) measured on 10 consecutive unfixed sections at Karl Zeiss (Oberkochen, West Germany), using transmitted light interference microscopy after Jamin-Lebedeff's method, varied from $0.5\text{--}1.1\text{ }\mu\text{m}$ (average $0.7\text{ }\mu\text{m}$). To achieve such thin sections of kidney tissue only new or newly sharpened knives (type C) are used (Larsen 1978a), and it is necessary to have permanent staff in charge of cutting. The advantage of using $1\text{ }\mu\text{m}$ cryostat sections of kidney tissue for IFM is that the appearance and localisation of the deposited antigen in the tissue can be evaluated more precisely than is possible with thicker sections (Larsen & Brun 1979).

Fixation

Fixation of kidney tissue for IFM is a balance between, on the one hand, preserving the antigens in the tissue and, on the other, not destroying, deforming or blocking the antigenic determinants. IFM can routinely be carried out on unfixed material. The most used fixative is 93% or 95% alcohol at room temperature or dry acetone at 4°C (Larsen 1978a, Nairn 1969, Evans et al. 1973). Fixation results in the structure of the kidney tissue being more clearly delineated. IFM on alcohol-fixed tissue, blocked in paraffin, is described by Sainte-Marie (1962). The method is particularly suitable for the demonstration of intracellular antibody (Johnson et al. 1967) but its use has also been reported in renal biopsies (Heron 1970). The advantage of this method is better preservation of morphology than is seen with cryostat sections and also the possibility of conventional histology from the same block. However, the identification of C is not optimal, and the demands which are nowadays placed on the histological preparation for diagnostic use in kidney disease using LM cannot be fulfilled with alcohol fixation. Other immunohistochemical methods (peroxidase–antiperoxidase (PAP) technique) used on formalin-fixed tissue blocked in paraffin (MacIver et al. 1979) are therefore preferable, though with the reservation that the number of positive findings can be variably dependent on the fixative used (Jacobsen et al. 1980).

Storage

An ideal method of storing frozen tissue is to place it in liquid nitrogen (– 196°C) in a Dewar container. By this means tissue can be preserved unaltered for several years (Wick et al. 1978). To store tissue in an ultra-deepfreeze (– 80°C) or in an insulated dry ice container (– 79°C) lined by plastic foil is more simple, more practical and generally in widespread use. Without special measures against dehydration (freeze drying) the tissue can be kept for less than 1 year by this method (Wick et al. 1978). This finding is in accord with the author's own experience (unpublished results). If the block of tissue is protected against dehydration by placing it in an airtight container or bag it can be stored for several years without alteration in morphology and antigenicity (Nairn 1969). To prevent drying during storage in an ultra-deepfreeze or container with dry ice, special bags were produced (120 × 90 mm) enlisting industrial help (Otto Nielsen's Emballage, Lyngby, Denmark). The sides of the bags have three layers, aluminium foil on the inside is

coated by polyethylene and on the outside polyethan. The bags are closed airtight by an "impulse welding apparatus" (made by Bosch). The author's own studies with IF carried out on renal biopsies stored by this method in dry ice containers over an observation period of 16 months, showed no change of morphology, identification of antigen, activity of fluorescence or localisation of antigen (unpublished results) when compared with the initial studies. Storage of tissue in an ordinary deepfreeze (about -20°C) allows it to be kept only a few weeks (Wick et al. 1978).

STAINING METHODS

Immunofluorescence technique

IF can be used in two ways: the direct or indirect technique. In the direct technique (one layer technique) the fluorochrome conjugated antibody is applied directly to the section of tissue, and so the corresponding antigens are demonstrated directly by binding to the conjugated antibody. This technique is the one which is most used. It is easy to carry out and precise localisation of the antigen in the tissue is possible (Larsen & Brun 1979), and the result of the investigation can be available in less than 3 hours (Larsen 1978a).

In the indirect technique (sandwich technique) the tissue section is covered initially with a layer of a specific, unconjugated antiserum so that antibody molecules will be bound to the corresponding antigen. The now deposited antibody molecules will act as antigens to a corresponding conjugated antiserum as in the direct technique. An advantage of the indirect technique is its 4–12 times greater sensitivity (Nairn 1969), for each primary bound antibody molecule binds a greater number of conjugate molecules so that the intensity of fluorescence is increased proportionally with the number of conjugate molecules. A further advantage is that the same conjugate can be used together with many different antibodies from the same type of animal as the conjugate is directed against.

Comparable studies of the direct and indirect technique used on kidney tissue from patient with GN (Tarantino et al. 1973) have shown almost the same frequency in the demonstration of Ig (G, M, A), C_3 , C_4 , C_{1q} and fibrin. In contrast, IgD could only be demonstrated by the indirect technique and the frequency of IgE using this technique was found to be double that seen with the direct technique. The indirect technique is thus preferable for identification of the deposited immune

reactants but in contrast the localisation of these deposits is less precise. Optimal identification and localisation of deposits demands that both the direct and indirect techniques are used.

Immunofluorescence microscopy

IFM is a complex immuno-optical system which like histochemical staining methods is characterised by its high specificity and sensitivity. The specificity depends on a biochemical reaction between the immune sera used and the corresponding antigens in the tissue. This is a process which is in itself not visible and therefore an indicator system is needed. Different stains (fluorochromes) can be used for this purpose, which can be coupled (conjugated) to antibodies so that these can be observed in a special optical system (Nairn 1969) which makes the sensitivity high. Fluorochrome-conjugated conjugates (lyophilised or in solution) can be stored at temperatures -20°C to 5°C and will keep for about 3 years (Green et al. 1976).

In spite of the development and improvement of fluorochromes, purification of antibodies, methods of conjugation and improvement in filter technique and the optical systems which have occurred in recent years, it must be realised that results of IFM will never be better than the individual components which are involved in this complex method. Details about these problems are reviewed by Sternberger (1979). Fluorescence is one property of fluorochromes which is characterised by them giving off light (emission) of another wavelength than the one which they have absorbed following radiation (excitation). The emission of fluorochromes will always be of a greater wavelength than that which is absorbed at excitation (Stokes Law) (Wick et al. 1978).

Among the many fluorochromes which can be used (Nairn 1969) fluorescein-iso-thio-cyanate (FITC) has been widely employed. One contributory reason for this is presumably the large supply of a broad spectrum of different FITC conjugated immune sera which have been commercially available in recent years. The advantage of FITC is a brilliant green emission with a wavelength that coincides with maximal spectral sensitivity of the eye (Tomlinson 1972). One disadvantage of FITC, with the energy sources which are nowadays used for excitation, is a rapid decline in the intensity of fluorescence (fading) which can reduce to 50% of its initial level after 2 seconds of excitation with a XBO₇₅ xenon lamp (Enerbäck & Johansson 1973). Rhodamine conjugates with their greater resistance to fading have therefore been suggested as

an alternative to FITC (Brandtzaeg 1975). Rodamine conjugates can be used together with FITC conjugates for simultaneous demonstration of two different antigens with the same localisation in tissue preparations (Kim et al. 1979). The energy source which is used most for excitation is HBO 200W or 100W high pressure mercury vapour lamp. These lamps are expensive to use because the optimal energy spectrum for excitation of FITC has only a life of 50–75 burning hours. Various efforts to find other systems of illumination for IFM have shown that both iodine quartz lamp (Heimer & Taylor 1972) and tungsten halogen lamps (Rygaard & Olsen 1969) can be used if optimal interference filters are used.

Experimental studies with an energy-rich monochromatic emission using an argon laser for excitation of FITC conjugates, has demonstrated the intensity of the fluorescence is 100 times greater than with the conventional lamps. There is recovery of the initial fluorescence after 48 hours storage in the dark (Kaufman et al. 1971), and quantitative estimations of the intensity of the fluorescence is possible using a laser as the light source (Berns 1979).

The optical system of the fluorescent microscope for use with transmitted and incident light and the arrangement of the filter systems are described in detail by Nairn (1969). Transmitted light is used mostly for IFM with a darkground condenser of emmersion type. It is useful in routine microscopy to use a toric lens attached under the condenser (Tiyoda) for this produces illumination of a large area in the object examined. The use of this is limited by the magnification (Max. $\times 25$ objective). For more exact work using objectives over $\times 40$ the conventional darkground condenser is used (Nairn 1969).

Choice of the excitation filter (for transmission of the required wavelength for irradiating the fluorochrome) and the barrier filter (for cutting out the excitation light) for transmission of the emission of the fluorochrome is important, for the absorption maximum of FITC (490 nm) lies close to the emission curve (515 nm). The type of interference filters, which is used nowadays, give a good separation between the light of excitation and emission (Tomlinson 1972), which together with a high transmission of the fluorescence gives high sensitivity, though this varies with the different makes of interference filter (Herzog et al. 1973). Interference filters which have transmission of a narrow red band, above the wavelength of the excitation light (Rygaard & Olsen 1969), gives a marked contrast between the green brilliant fluorescence and the tissues red background colour. This makes it possible to pin-

point the localisation of the fluorescing areas in the tissue (Larsen 1978a).

In addition autofluorescence is reduced to a minimum by using these filters (Rygaard & Olsen 1969, Herzog et al. 1973).

Assessment of the intensity of fluorescence

Quantitative IFM for assessing the amounts of deposited antigen in tissue does not exist and measurement of the intensity of fluorescence by microfluometry (Nairn 1969) is more an expression of the reagents used, the optical system and light source, than the deposited amounts of antigen.

The intensity of fluorescence is usually expressed semiquantitatively on an arbitrary 4 point scale where 0 or – (= negative) represents the investigator's attitude to lack of demonstration of antigen, whilst +++ indicates the optimal intensity of fluorescence. The intensity of fluorescence can be an expression of the greater or lesser amounts of deposited antigen but the use of weak sera, over- or under-conjugated conjugates, pH, light source, filters (Nairn 1969) and adaption to the "dark" do play a role. These factors can be very crucial in the borderline area "no fluorescence" or "lowest degree of intensity of fluorescence" when comparing the number of positive and negative results of antigen findings in different studies. It must also be presumed that in this borderline area subjectivity plays a role in the IFM result if the clinical and morphological information is known prior to the IFM investigation. Because of this the author's investigations were assessed without clinical or morphological information being available to him at the time of the study (Larsen 1978a, Larsen 1978b, Larsen 1979, Larsen & Brun 1979).

Immunoperoxidase technique

Enzymes such as horseradish peroxidase (HRP) can be conjugated with antisera in a similar way to fluorochromes without the immunological activity being lost (Nakane & Pierce 1967). HRP conjugates can be used in direct and indirect techniques in the same way as fluorochrome conjugates (Sternberger 1979). Comparable investigations of IF and immunoperoxidase methods performed on cryostat sections of renal biopsies have shown results which are in agreement (Davey & Busch 1970, Turner et al. 1979). The method used on formalin fixed tissue blocked in paraffin is suitable for demonstrating intracellular antigens (Taylor 1978) in contrast to extracellular antigens such as Ig and C as

reported by MacIver et al. (1979). This is in agreement with the author's own studies on renal biopsies (unpublished results). On the other hand, extracellular Ig and C can be demonstrated using a new method (PAP) (Sternberger 1979). The method, when used on formalin-fixed paraffin-blocked tissue from renal biopsies following 40 minutes incubation with 0.05% trypsin, has shown a good correlation with conventional IF performed on frozen renal tissue (MacIver et al. 1979).

The advantages and disadvantages of immunoperoxidase methods compared with IF methods have been recently reviewed by Taylor (1978), and the importance of the fixation to the frequency of positive findings in the peroxidase technique is reported by Jacobsen et al. (1980).

Electron microscopy study

In immunological studies run in parallel with ultrastructural investigation of kidney tissue in immune complex nephritis (Germuth et al. 1979), nephrotoxic serum nephritis (Feldman et al. 1963) and autologous immune complex nephropathy (Heymann nephritis) (Couser et al. 1976) it is possible to demonstrate electron dense deposits in the GBM during the course of the disease. Sequential IFM and EM studies of autologous immune complex nephropathy (Couser et al. 1976) have shown that electron dense deposits together with complement were first visible on EM one week after deposited IgG was demonstrated on IFM. IFM was thus found to be more sensitive than EM with early deposition of immune deposits.

Whether subepithelial dense deposits – “humps” (Kimmelstiel et al. 1962) or intramembranous or subendothelial electron dense deposits in the different types of human GN, are always an indication of Ig and/or C deposition is still not clarified with certainty. Dense deposits are also seen after administration of heavy metals such as gold and iron (Churg & Grishman 1972). Thus it is not possible to exclude that the electron-dense areas in both the GBM and mesangium are on expression of altered basement membrane metabolism (synthesis and/or degradation) induced, for example, by immune complexes and thus not recently an indicator of deposits themselves (Kim et al. 1979).

Even though to day it is broadly accepted that Ig and C seen on IFM correspond to electron-dense deposits in glomeruli demonstrated by EM, direct proof that this is the case can only be settled by immuno-EM, which is nowadays possible using HRP conjugated antisera (Davies et al. 1977).

V CLASSIFICATION OF IMMUNOFLUORESCENCE MICROSCOPY FINDINGS IN GLOMERULI

In recent years development in the treatment of both acute and irreversible terminal renal failure has created increasing interest in, and need for, more precise diagnosis. This has resulted in a remarkable advance in the investigative methods used to study the morbid anatomy and immunopathology of renal disease so that a more precise diagnosis is obtained. This applies of course to all groups of renal disease but particularly to GN, where nowadays it is generally accepted that most cases are caused by immunopathogenetic mechanisms which to a certain extent are analogous with experimental models of GN (Dixon 1968). The most commonly occurring is immune complex nephritis, where circulating antigen-antibody complexes deposit in glomeruli and can be demonstrated by IFM as granular deposits in the wall of the capillary loops and/or mesangium.

Much more rare is the appearance of anti-GBM nephritis (linear nephritis) which is caused by circulating antibodies reacting with GBM. With IFM these can be demonstrated as continuous linear deposits.

Clinically and on LM it is difficult, and in some cases impossible to differentiate between these different immunological mechanisms. A differential diagnosis can only be made using immunomicroscopy.

Demarcation and classification of human GN is nowadays based on clinical and morphological criteria (Pirani & Salinas-Madrigal 1968, Habib 1970). In spite of there being well defined lesions in glomeruli as a basis for subdividing GN into different types, there is no specific relation between uniform morbid anatomical changes and the clinical picture (Cameron 1975). It is thus difficult to explain just how the two mechanisms can induce such widely different morphological lesions in glomeruli, which re-biopsy has demonstrated do not represent different stages in the same glomerular disease (Larsen & Brun 1979).

An immunopathological classification, based on an analysis of the composition of antigen-antibody complexes, their appearance and localisation in glomeruli, could therefore be considered to be either an

alternative to the morphological classification or a supplement to it, to achieve a more exact description of GN in groups of patients with uniform LM changes.

In contrast to experimental GN the antigen in the deposited antigen-antibody system in glomeruli in human GN is seldom known. Immune complexes in human GN can thus only be identified from the presumed antibody part of immune complexes and accompanying immunoreactive serum proteins.

Routine use of IFM on renal biopsies from patients with GN is therefore based on an identification of the composition of the deposits – the different classes of Ig (G, M, A, D, E), C fractions (C_{1q} , C_4 , C_3), properdin and fibrin/fibrinogen:

CLASSIFICATION MODEL OF IMMUNOFLUORESCENCE MICROSCOPY FINDINGS IN GLOMERULI

Classification model

A classification model of immunopathological findings in glomeruli based on the composition of the deposits, their pattern and localisation in glomeruli is given in fig. 1–3.

Fig. 1 (a–e) shows potential possibilities of IFM findings grouped according to the composition of the Ig classes, C fractions and properdin (a–b), Ig without C (c), C without Ig (d) or without demonstrable deposits = “no deposits” (e).

Deposits of fibrin/fibrinogen are not in the classification but can appear in all the groups mentioned.

Fig. 2 illustrates a schematic representation of the renal corpuscle with the structures named (arrows). A–D illustrate the pattern and localisation of deposits in glomeruli.

Pattern of deposits:

The pattern of deposits may be *granular* (A–C) or *continuous linear* (D).

Localisation of deposits:

Granular deposits can be localised solely in or along the GBM (A), solely in the mesangium (B), or both in the mesangium and along the GBM (C).

**Aspects of a Classification of Immune Deposits,
Pattern and Localisation in Glomeruli**

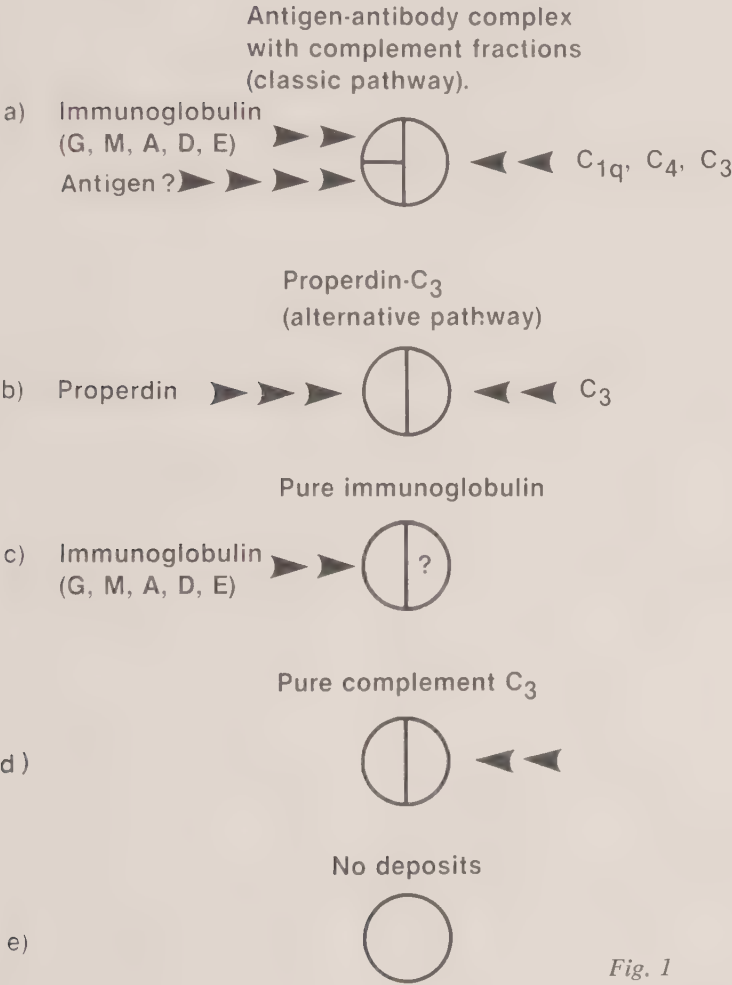
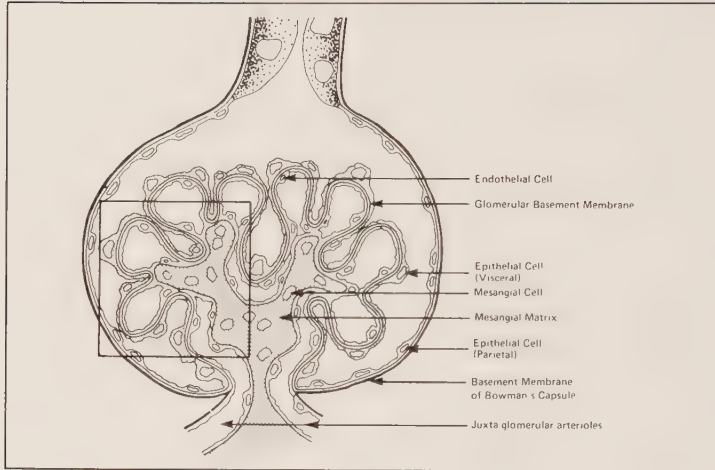


Fig. 1

THE RENAL CORPUSCLE



A-D: PATTERN AND LOCALISATION OF IMMUNE DEPOSITS IN THE GLOMERULUS

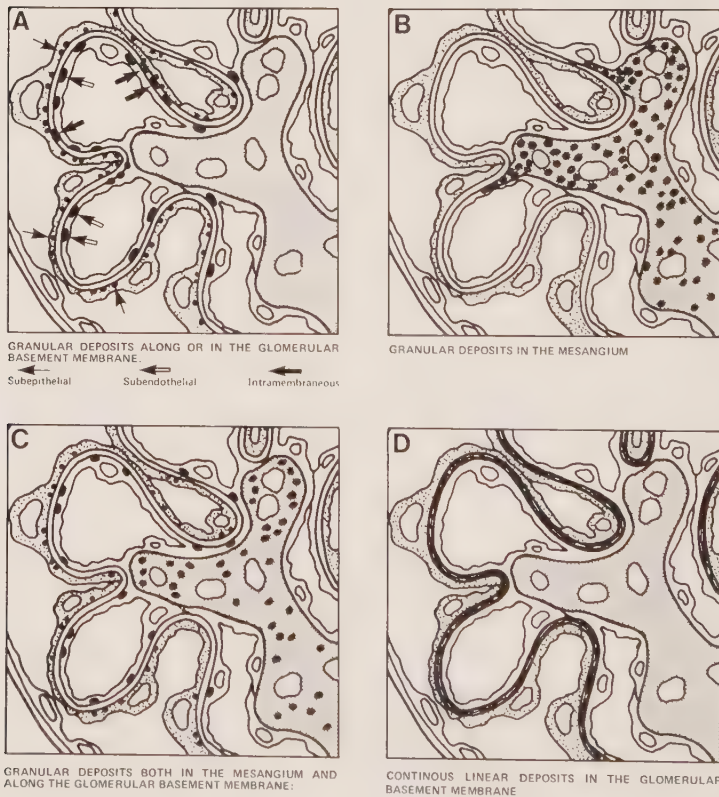
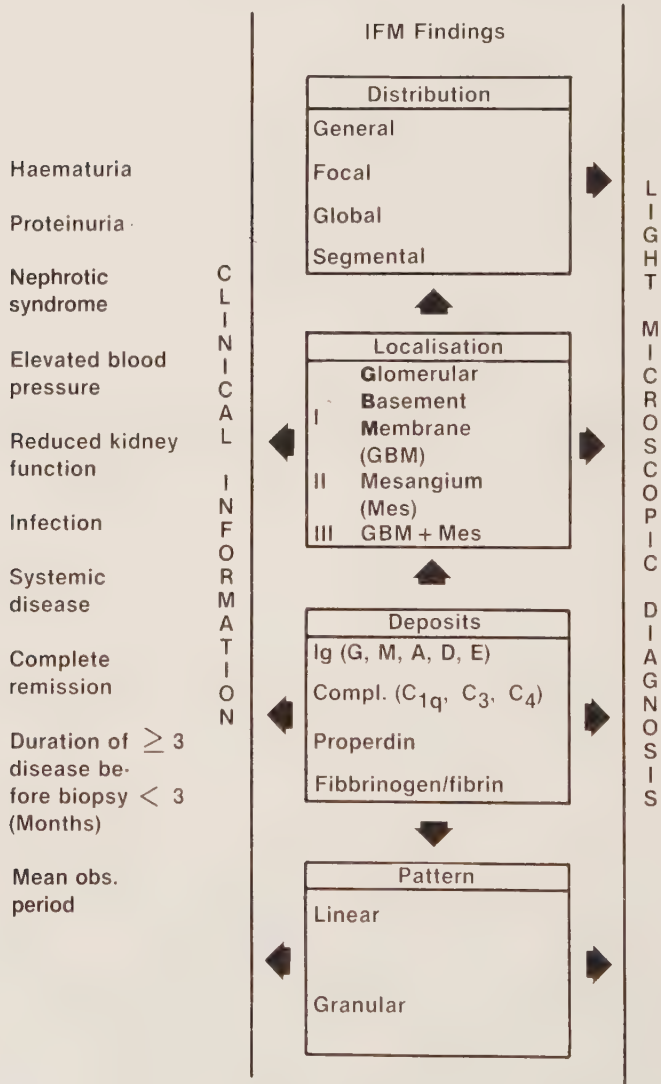


Fig. 2

Association of IFM Findings with LM Diagnosis and Clinical Observations.



IFM = Immunofluorescent microscopy *Fig. 4*

VI CLASSIFICATION OF HUMAN GLOMERULONEPHRITIS BY IMMUNOFLUORESCENCE MICROSCOPY (*Author's own studies*)

IFM findings in glomeruli of kidney tissue from 395 patients (referred to in fig. 5) are correlated to the LM and clinical information in order to investigate:

1) Correlation between IFM findings and the clinical symptoms, the clinical course of the disease and response to immunosuppressive therapy, and whether IFM findings could significantly define different groups of patients with GN,

a) partly independent of the morphological type of lesion in glomeruli (Larsen & Brun 1979),

IFM investigations of 422 Kidney specimens from 395 patients.

Classifications (Combined LM and clinical criterial)	Patients	Biopsies	Re-biopsy	Autopsy	Reference article
	No.				
Glomerulonephritis (GN)	290	290	26		Larsen 1978a Larsen 1978b Larsen & Brun 1979
Non-glomerulonephritic nephropathy	49	49	1		Larsen & Brun 1979
No nephropathy	56	23		33	Larsen 1979
Total	395	362	27	33	

Fig. 5

- b) partly in the individual groups of patients with the same morphological changes on LM (Larsen 1978a, Larsen 1978b, Larsen & Brun 1979).
- 2) In what way IFM findings in glomeruli of patients with non-glomerulonephritic nephropathy differ from those presenting with GN classified by LM (Larsen & Brun 1979).
- 3) The possible frequency and composition of Ig and C fractions which can be expected to occur in people with no clinical or morphological evidence of glomerulopathy (Larsen 1979).

Statistical methods

All correlations between the IFM findings and the clinical symptoms and the clinical course were tested by using the Chi square test or, in the case of low expected frequencies, the Fisher exact probability test (Siegel 1956) was used.

IMMUNOFLUORESCENCE MICROSCOPY FINDINGS IN GLOMERULI IN 290 RENAL BIOPSIES/CORRELATED TO LIGHT MICROSCOPY AND CLINICAL DATA AS WELL AS THE MORPHOLOGY, DISTRIBUTION AND LOCALISATION OF IMMUNOREACTANTS

The composition of the deposits with the different classes of Ig (G, M, A), with and without C₃, appear in table 2 (Larsen 1979).

Ig/C₃ occurred in 74%. Either Ig (6%) or C₃ (7%) or "no glomerular deposits" (13%) were found in the remaining 26%.

Immunofluorescence microscopic correlations to light microscopy

No combinations of the deposited classes of Ig, with or without C, or "no deposits", were specifically correlated with the LM diagnoses. An increased frequency of IgA/C₃ and IgG-IgA/C₃ were demonstrated in "normal glomeruli" and in mesangioproliferative GN, respectively. There was an increased frequency of "no deposits" demonstrated in "minimal change lesion". Apart from this no significant correlation was found between the deposited classes of Ig and the LM diagnoses in GN.

Relation between deposited classes of immunoglobulin and C₃ and the clinical data (independent of light microscopic diagnosis)

Correlation of the demonstrated deposits to the clinical data appears in table 4 (Larsen & Brun 1979). With IgG-IgA/C₃ an increased frequency of haematuria and a decreased frequency of reduced renal function was found, and with IgA/C₃ infection was seen more frequently.

With IgM/C₃ there was less tendency for haematuria, and the duration of the disease prior to biopsy was at least 3 months. In "no glomerular deposits" there was an increased frequency of nephrotic syndrome.

Apart from this no significant correlations were found between the composition of deposited classes of Ig/C₃, the symptoms and the clinical course. This is in agreement with work reported by others (Donadio et al. 1978, Gärtner et al. 1979).

It was thus not possible to define specific categories of patients from the combination of deposited classes of Ig and C₃ (Larsen & Brun 1979).

Relation between deposited classes of Ig/C₃ and localisation in glomeruli

The relation between deposited classes of Ig/C₃ and their localisation in glomeruli appears in table 4 (Larsen & Brun 1979) where the significant correlations are noted.

IgA/C₃ and IgG-IgA/C₃ were significantly, more commonly localised solely to the mesangium than other types of deposits.

IgM/C₃ was more commonly found localised solely to the basement membranes of the capillary loops.

The different localisation of IgA and IgM in glomeruli can be the result of the molecular size of Ig (Germuth & Rodriguez 1973), but IgA eluted from kidney tissue with such complexes has further shown biological properties of a weak reactivity to normal mesangial tissue (Lowance 1973). It may therefore be expected that IgA and IgM will always have preference for localisation in the mesangium and capillary loops, respectively. For this reason IgA and IgM could not be used as an indicator for the different antigens which can be present in the presumed immune complexes.

Immunofluorescence microscopy findings correlated to immunosuppressive therapy

Less than 2 months after biopsy 127 (43%) of the patients classified as having GN were on corticosteroid and/or cytostatic treatment (not randomised). Correlation between complete remission in the treated

and untreated patients showed that the group of treated patients had a significantly increased frequency of complete remission (Larsen & Brun 1979, tab. 5). In contrast no significant difference in complete remission was found between the groups of treated patients with deposits of Ig/C₃ and those without these deposits. The same held true in the groups of patients who were not given therapy.

Thus treatment had a significant effect but this effect was independent of whether there were glomerular deposits of Ig/C₃ present or not.

The same applied in patients with the same morphological type of lesion (generalised mesangioproliferative GN) (Larsen 1978b, tab. 7).

Relations between the deposited classes of immunoglobulin/C₃ and the clinical data (in the individual light microscopic groups of glomerulonephritis)

In the individual groups of GN classified by LM, "normal glomeruli", "minimal change lesion", slight mesangioproliferative GN (Larsen 1978a), generalised mesangioproliferative GN (Larsen 1978b) and remaining LM types of GN (Larsen & Brun 1979) only a few significant correlations were found between the deposited classes of Ig (G, M, A) as well as combinations of these and the clinical data.

None of the demonstrated deposits could delineate specific categories of patients with uniform symptomatology and clinical course.

With mesangial deposits of IgA/C₃ or IgG-IgA/C₃ several patients with "normal glomeruli" (Larsen 1978a) and generalised mesangioproliferative GN (Larsen 1978b) had a clinical picture like IgA nephropathy (Berger & Hinglais 1968, Berger 1969). In some cases the same deposits were demonstrated in patients with HSP and in patients with severe proteinuria and nephrotic syndrome. Thus with IFM it is not possible to separate these categories of patients with certainty and a differential diagnosis is only possible when the IFM and clinical information is considered as a whole.

Immunofluorescence microscopic findings in re-biopsies

The IFM findings in 27 re-biopsies from 24 patients (Larsen & Brun 1979, tab. 6) showed:

- 1) that the LM morphological classification of GN does not alter during the course of the disease.
- 2) that Ig deposits were not always accompanied by C₃ in the period of observation.

- 3) that negative IFM findings in the first biopsy did not exclude the possibility that Ig and C₃ could be deposited later in the course of the disease.
- 4) that alterations in deposits (the different classes of Ig and C₃) during the course of the disease, were not related to the clinical course.
- 5) that neither positive nor negative findings give any direct information about the clinical course.

Distribution of the deposits

In all LM groups of GN the deposits were found to be generalised, except in a single case where they were focal.

Segmental deposits were demonstrated in occasional cases in all LM groups of GN, except in "minimal change lesion". Segmental deposits were often demonstrated where GN was secondary to SLE (26%).

In the remaining biopsies the deposits were global.

Pattern of the deposits

In patients classified as having GN 5 cases (2%) were found to have continuous linear deposits of Ig/C₃ localised solely to the basement membranes of the capillary loops (anti-GBM nephritis). Granular deposits of Ig/C₃ in glomeruli (complex nephritis) were demonstrated in 210 patients (72%).

Very uniform fine granular deposits along the basement membranes of the capillary loops were characteristic of epimembranous GN. The most coarse granular deposits were found in lobular GN and MPGN. In all other cases the deposits had a mixed fine and coarsely granular appearance.

Discontinuous linear deposits could be seen together with granular deposits which were peripherally placed in a few of the capillary loops in proliferative GN of lobular type, MPGN and in non-classifiable GN (35–40%) and in other classes of GN (less than 27%).

No correlation was found between discontinuous linear deposits and the duration of the disease.

Glomerular deposits of immunoglobulin/C₃ in glomerulonephritis secondary to "systemic disease" (SD)

Of the 290 patients classified as having GN there were 47 cases (16%) secondary to SD. The relations between the IFM findings, LM and clinical symptoms appear in table 7 (Larsen & Brun 1979).

The deposits, as judged from the different classes of Ig and their composition with or without C_3 , did not differ from the deposits which could be demonstrated in primary GN. However, the composition of Ig (G, M, A)/ C_3 predominated.

Continuous linear deposits of IgG/ C_3 in GBM (anti-GBM nephritis) were demonstrated in 4 cases (9%) in this group. Circulating antibodies directed against GBM were demonstrated in one case (periarteritis nodosa), but in contrast these antibodies were not demonstrated in 2 patients with a clinical picture like that of Goodpasture's syndrome (Duncan et al. 1965).

Immune complex nephritis characterised by granular deposits of Ig/ C_3 was found in 39 patients (83%). In patients with SLE and HSP the distribution was characteristic. In SLE IgG and IgM had a global distribution, whilst IgA could be demonstrated as segmental discontinuous linear deposits lying peripherally in the basement membranes of a few of the capillary loops. In HSP the deposits were IgG-IgA/ C_3 , generalised and global, and mainly localised to the mesangium but in addition were seen out along the basement membrane of the capillary loops near the mesangium.

*The frequency of classes of immunoglobulin in:
Glomerulonephritis*

The frequency of the different classes of Ig in GN (secondary to SD) were as follows: IgG = 74%, IgM = 47%, IgA = 55%, where the highest frequency of IgA appeared in SLE (60%) and in HSP (88%). In GN (without SD) the frequency of the classes of Ig were as follows: IgG = 62%, IgM = 44% and IgA = 41%.

Non-glomerulonephritic nephropathy

IFM investigations of renal biopsies from 49 patients with non-glomerulonephritic nephropathy (Larsen & Brun 1979) showed no significant correlations to LM diagnoses or clinical data. Glomerular deposits with the globulin composition IgA/ C_3 and IgG-IgA/ C_3 could not be demonstrated.

The frequency of the different classes of Ig were as follows: IgG = 24%, IgM = 20% and IgA = 10%.

Immune deposits in glomeruli in patients without clinical or morphological evidence of glomerulopathy

IFM was carried out on kidney tissue from patients with normal blood pressure and without clinical or histological evidence of glomerulopathy.

This group thus forms a "normal population" and made it possible to investigate the occurrence and quality of immune deposits in such a situation.

The study included kidney tissue from 56 patients of which 23 were renal biopsies from patients on lithium treatment (included in a current study on this specific problem), and 33 were carried out on autopsy material.

Deposits of IgG, IgM and C fractions were demonstrated in 33% of the 56 patients. IgA could not be demonstrated. The distribution of these in the biopsies and the autopsy material appear in table 2 and table 3, respectively (Larsen 1979).

The distribution of the deposits was generalised and they had an appearance and localisation which did not differ from that seen in immune complex nephritis. Similar findings of glomerular deposits of IgG, IgM and/or C₃ have been reported in patients without evidence of kidney disease by other workers (Sutherland et al. 1974b, Velosa et al. 1976) just as non-specifically bound IgG has been demonstrated in eluate from normal kidneys in older patients without evidence of kidney disease (Wesenberg & Tönder 1978).

It can thus not be excluded that these deposits of IgG, IgM and C fractions demonstrated in the material presented here, come close to representing the frequency of glomerular deposits which can be demonstrated in normal persons. The deposits are possibly the result of a glomerular clearance function which plays its part in the body's reticulo-histiocytic system (Zollinger 1978) in eliminating circulating non-nephritogenic immune complexes produced by day to day infections to which the body can be exposed. If such "spontaneous" deposits of immune complexes are a relatively normal occurrence, then these deposits will necessarily be demonstrated by IFM in kidney biopsies in some cases of human GN, even though they have no relation to the disease. Such deposits of immunoreactants in glomeruli can be a contributory cause of the lack of correlation of the immune deposits to the histological and clinical findings in GN.

VII COMMENTS

In this investigation of IFM on renal biopsies from 290 patients classified on LM as having GN (including "normal glomeruli" and "minimal change lesion"), 2 characteristic patterns of deposited Ig/C₃ were demonstrated in glomeruli, corresponding to the 2 main types of immune mechanisms which are generally accepted as pathogenetic in most cases of human GN (Dixon 1968, Wilson & Dixon 1974a). Linear nephritis (anti-GBM nephritis) was demonstrated in 5 patients (2%). The incidence of linear nephritis is thus relatively rare and geographical differences do not seem to be relevant (see page 21). The importance of circulating antibodies in the induction of anti-GBM nephritis has been demonstrated (Lerner et al. 1967) and a renal lesion is well known as the basis for Goodpasture's syndrome (Duncan et al. 1965, Koffler et al. 1969).

Two of the 5 patients in this study had Goodpasture's syndrome. To prove this with certainty requires demonstration of circulating antibodies to GBM in the patient's serum (Dixon 1968). This could not be demonstrated in these 2 patients (incomplete Goodpasture's syndrome). This does not, however, exclude the diagnosis as being correct for the concentration of serum anti-GBM Ig often first rises to an identifiable level after nephrectomy (Lerner et al. 1967). In 3 patients with linear nephritis haemoptysis did not occur but in one of these (with periarteritis nodosa) circulating anti-GBM Ig was demonstrated. The same IFM findings of linearly deposited IgG/C₃ in the basement membranes of the capillary loops in these 5 patients give a suggestion of a common pathogenesis. These 5 patients were at the same time divided into 4 different LM groups of GN and they demonstrated different extrarenal manifestations which suggest a heterogenous pathogenesis.

IFM can thus bring linear nephritis to light but an exact diagnosis can only be achieved from further information about the morphological findings and the clinical data.

The deposition of circulating antigen-antibody complexes in glomerular tissue is the immunopathogenetic mechanism (McCluskey 1970) which is nowadays generally accepted as the most common cause of human GN. In the study presented here granular deposits of Ig/C₃ were

demonstrated in glomeruli in 210 patients (72%). These deposits occur in both primary GN and GN secondary to SD, as reported by other workers (Michael et al. 1966, Hyman et al. 1973).

The aetiology was unknown in 70% of the 210 patients with complex nephritis, whilst there was clinical "evidence" that streptococci were the aetiological factor for GN in 30% of cases. However, the same incidence of information about streptococci appears in the group of 49 patients with non-glomerulonephritic nephropathy. The incidence of presumed streptococcal-induced GN must therefore be presumed lower than would be expected from the clinical data, in spite of antibiotic treatment having been previously given. This is in agreement with what has been previously reported in GN in children (Meadow 1975).

Glomerular deposits of different classes of Ig and their combinations in GN were not specifically related to any type of glomerular lesion, clinical symptoms or the clinical course. The clinical picture which could be seen in patients with deposits of Ig/C₃ was also found in patients without deposits.

The different IFM findings in the individual morphological types of GN therefore suggest a heterogenous pathogenesis, where the Ig fraction in the presumed immune complexes could not be shown to have any prevailing influence on either the type of glomerular lesion or on the nature of the symptoms or the clinical course – findings which are in agreement with reports by other workers (Gärtner et al. 1979, Donadio et al. 1978).

The lack of correlation of the IFM findings to LM and the clinical picture can be due to different causes:

- 1) there was agreement between LM diagnoses on the first biopsies examined and the later re-biopsies, but in several cases no immune deposits were found where the biopsy was obtained less than 3 months after the disease was proved. However, immune deposits were demonstrated later in the re-biopsies from some of these patients. The same situation must be presumed to prevail in other patients in the study even though this was not confirmed by re-biopsy. These deposits can thus represent a secondary event resulting from secondary deposition of non-nephritogenic immune complexes in the preceding lesions, possibly as a result of normal glomerular clearance of immune complexes from the blood serum (Larsen 1979).

- 2) As shown in the re-biopsies a change in the composition of the deposited classes of Ig can occur in the course of disease. This can re-

sult in IFM findings varying significantly in the same patients dependent on the timing of the biopsy in relation to the start of the disease, which is of course not always known and not necessarily coincident with the first appearance of symptoms of the disease.

Deposits of IgA/C₃ and IgG-IgA/C₃ were significantly more commonly localised solely to the mesangium than other types of deposits, and IgM/C₃ was found significantly correlated to the basement membranes of the capillary loops. This significant difference in localisation of IgA and IgM may be due to the size of the Ig molecules (Germuth & Rodriguez 1973) and in addition it has been demonstrated that IgA as an antibody has weak reactivity against normal mesangial tissue (Lowance 1973). These physiological and biological properties of Ig can signify that they will always be preferentially localised to mesangium and capillary loops, respectively. Thus, these deposits cannot be used as an indicator for possible different antigens which must be presumed to be represented in the presumed immune complexes.

The immunohistological picture of IgA-IgG nephropathy (Berger & Hinglais 1968) is not found to be associated with any uniform or characteristic clinical and morphological picture. Mesangial deposits of IgA/C₃ and IgG-IgA/C₃ cannot therefore be regarded as a clinical/morphological entity, but rather as a specific immunohistological clinical sign with different underlying pathological mechanisms which are possibly controlled by a common, genetically determined aberrant immune response (Sabatier et al. 1979), with varying morphological reactions of different degrees of severity and a relatively favourable prognosis (Gärtner et al. 1979).

IgA was demonstrated in GN (without SD) in 41% of cases. In GN (secondary to SD) IgA was found in 55%, with the highest frequency in SLE (60%) and HSP (88%). IgA was demonstrated in only 10% of patients with non-glomerulonephritic nephropathy and not at all in a "normal" population. The demonstration of IgA is therefore a good indicator for confirming the diagnosis GN made on LM. In addition, the appearance of the deposits and their localisation can in some cases arouse suspicion that the GN can be secondary to SLE or HSP.

The demonstration of IgG and/or IgM in GN is not found to be sufficient evidence of GN, for these deposits could also be demonstrated in 40% of patients with non-glomerulonephritic nephropathy and in glomeruli in 1/3 of patients without clinical or morphological evidence of glomerulopathy.

VIII CONCLUSIONS

Deposits of different classes of Ig and their combination could therefore be used as a basis for an immunopathological classification of patients with GN at LM. However, such a classification seems only to be another descriptive level with the same correlative problems as the LM diagnoses.

IFM examination of renal biopsies classified as GN on LM will not give conclusive information about:

- 1) the clinical symptoms
- 2) the prognosis
- 3) indication for or against the use of immunosuppressive therapy
- 4) the effect of immunosuppressive therapy.

Furthermore

- 5) deposits of IgG and IgM in glomeruli are not necessarily evidence of nephritogenic complexes.
- 6) deposits of C₃ in its own are not proof of an immune complex nephritis.
- 7) "no deposits" do not exclude the possibility of GN on LM.

Possible diagnostic information which can be obtained by IFM examination of glomeruli in renal biopsies classified on LM as GN.

- 1) demonstration of antigen material

bacteria
viruses
components of host tissue
- 2) demonstration of linear nephritis.
- 3) demonstration of most cases of epimembranous GN still not visible on LM ("minimal change lesion").
- 4) call attention to the possibility of GN being secondary to other primary disease.

- 5) form a basis for the classification of patients where IgA (IgG-IgA) is deposited solely in the mesangium.
- 6) demonstration of IgA supports the light microscopic diagnosis GN.
- 7) demonstration of C₃ activation via
 - a) the classical pathway and
 - b) the alternative pathway.

IFM findings are thus, in some cases, a necessary supplement to the morphological and clinical data for achieving a practical diagnostic demarcation of categories of patients within the somewhat difficult-to-define concept which today constitutes GN.

IX PERSPECTIVE

Lengthy investigations based on re-biopsies can, if this is a practical possibility and ethically defensible, probably give better correlation between the IFM findings of Ig in glomeruli and the relation of C profiles to the prognosis in GN. However, focusing on the identification of the involved antigen in immune complexes (both in glomeruli and the circulation) must be accepted as being more practicable of obtaining further information and understanding of the basic causes for induction and the clinical course of the disease in GN, compared with what nowadays may be deduced from glomerular deposits of Ig, C, and fibrin.

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